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Beneficial Effects of Soybean and Cassava in Local Wisdom Feed Diets on Growth Performance with Nutritional and Economic Analysis of Snakehead Fish (*Channa Striata*)

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Abstract: Substituting plant protein for fish meals is an alternate protein source in the diet of reared fish. By replacing fish meal with Soybean Meal (SM) or Cassava Meal (CM), this study determined the effects of diets influenced by local wisdom containing plant protein meal, SM, CM, or a commercial formula (FM^{TM}) on the survival and growth rates of snakehead fish (*Channa striata*) with nutritional and economic analysis. After acclimatization, snakehead fingerlings (10 fish/tank) were randomly allocated in the nine cement tanks. Three tanks each for Soybean Meal (SM) diet; Cassava Meal (CM) diet; and commercial diet (FM^{TM}). Fish weights were determined every four weeks for the 12 weeks. At the end of each experiment, the Survival Rate (SR), growth performance values, and cost reduction were calculated. After 12 weeks, there were no significant differences in SR among treatment groups. The growth performance of fish in SM dietary treatment and FM^{TM} dietary treatments had no significant difference. Although, the specific growth rate of fish fed CM diet was slightly less than SM and FM^{TM} diet .The soybean meal or cassava was substituted for a fish meal resulting in 11.6 and 7.9% economic value (cost/100g fish product), respectively compared to the commercial diet. However, the CM diet resulted in the lowest Feed Conversion Rate (FCR) in this experiment. There were no significant differences between FCR and Protein Efficiency Rate (PER). The results revealed the beneficial effect of diets influenced by local wisdom, especially the addition of soybean, in terms of weight gain promotion and feed cost reduction. These findings can be applied to develop low-cost diets for snakehead fish, supporting the flexibility and competitiveness of the snakehead fish farming industry and reducing the number of small fish used for fishmeal.

Keywords: Snakehead Fish, Growth Performance, Soybean Meal, Cassava Meal, Cost Reduction

Introduction

Snakehead fish (*Channa striata*) are economically important and popular in Thailand, Cambodia, Vietnam, and other Southeast Asian countries (Munir *et al*., 2016; Tu *et al*., 2022). Snakehead, a carnivorous freshwater fish, typically requires a high-protein diet, and Fishmeal (FM) is the main protein source for carnivorous fish in commercial feed (Riche, 2015). Fish diets are one of the important indicators of fish farming success. High-quality diets result in high growth and survival rates. However, high-quality diets do not guarantee good profits because high-quality feeds often have more expensive raw materials in their ingredients (Duan *et al*., 2022). So, farmers are often faced with the problem of raising costs, when FM resources are limited and the price of FM price kept rising in recent years (Thai Fisheries Department, 2019). The most pressing challenge to the aquaculture industry is cost reduction as far as possible by reducing feed costs. Substitution of FM protein with Soybean Meal (SM) protein in the fish feed is an option to promote raising snakehead fish as a sustainable career.

The replacement of FM by plant proteins in aquaculture feeds has been widely studied (Rombenso *et al*., 2013; Hien *et al*., 2016; 2017). Seminal contributions were made by Hien *et al*. (2016), which revealed that snakehead fish consuming a diet with 20% of SM showed greater growth and incurred lower feeding costs than those fed a formulated diet. The replacement of 45% FM with a combination of plant-based proteins, comprised of sunflower, cottonseed, linseed, canola, and sesame meals, did not significantly affect the growth performance of tilapia, *Oreochromis niloticus*. The production of juvenile hybrid striped bass tended to decrease in fish-fed SM diets comprising less than 10% FM (Rombenso *et al*., 2013).

Among plant-based fish meals, the promising effects of soybean and cassava have been documented. SM is the most common FM substitute in aquatic feeds because it has a high protein content, a good amino acid profile, and a comparatively low price and it is generally available (Miao *et al*., 2018). However, this plant-based meal contains substances that reduce nutrient utilization. For example, phytic acid, one of the anti-nutritive constituents in plants, bonds to divalent cations, which causes mineral deficiency in freshwater fish (Lall, 2002). Yaghoubi *et al*. (2016) have reported that SM and isolated soy proteins could substitute for up to 27.3% of dietary FM without negatively affecting the growth of juvenile silvery-black porgy. Moreover, Sagada *et al*. (2017) have reported that feed including 48% protein containing either 12 or 15% lipids was suitable for promoting the growth of juvenile northern snakehead fish (*Channa argus*). Thus, different species of fish adjust differently to soybean substitution. Cassava leaf has also been reported to be rich in protein, but it cannot exceed 20% as a supplement to fish diets because it contains toxic substances: Hydrogen cyanide, which can result in a malfunction of the central nervous system, and tannins, which can bind and inhibit endogenous proteins, such as digestive enzymes (Akande *et al*., 2010).

The present study considers the cost of new formulations based on folk wisdom with inexpensive local ingredients such as SM, cassava leaves, bananas, corn, acacia leaves, coconut meal, and golden apple snails (*Pomacea canaliculate*). This is the first time an alternative feed has been developed based on local wisdom for snakehead fish to reduce dependence on the high cost of fishmeal. The efficacy of diets that incorporate plant materials, specifically SM and Cassava Meal (CM), was determined and the costs were analyzed based on the costs of SM and CM vs. FM^{TM}).

Materials and Methods

Ethical Approval

The study was performed per the procedure accepted by the Srinakharinwirot University - Institute Animal Care and Use Committee (COA/AE-026-2563).

Fish Rearing

The experiment was conducted at a local fish farm in Mae La Subdistrict, Bang Rachan District. Singburi Province, Thailand (14°56'43.414N 100°19'58.801E) . A total of 150 snakehead fingerlings) 2–3 in (were obtained from a farm and cultured for 4 weeks in cement tanks (1.2 m diameter × 0.8 m height) on a commercial diet (43% protein and 6% lipids) to acclimatize. The water temperature and pH were noted twice a day. The survival rate was 82.67. After four weeks, 90 snakehead fingerlings (avg. wt. 10.61±1.42 g) were randomly allocated to tanks (10 fish/tank) in nine outdoor cement tanks with three replicates for each treatment (i.e., three tanks each for the SM diet, the CM diet and the commercial diet (FM^{TM})).

Experimental Diets

In this study, there were three experimental groups. The commercial diet, FM^{TM} (control), was obtained from the local farm mentioned above. Two local wisdom-influenced diets (SM and CM) were prepared at the Department of Biology, Thepsatri Rajabhat University. All experimental diets comprised 40% protein and 12% lipids and were stored in airtight polyethylene bags at room temperature. The formulations of the local wisdom-influenced diets are represented in Table 1.

Feeding Trial

The fish were fed until obvious satiety three times daily (8:00, 12:00, and 17:00). Leftover feed was eliminated and the feed consumption was calculated from the amount fed minus the amount left based on the dry weight. During the feeding trial, water temperature, dissolved oxygen, and pH ranged from 27.0–28.5°C, 5.0–7.6 ppm, and 7.0–7.2, respectively. The water in each tank was changed by 30% twice a week.

Growth Performance

Fish weights were determined every four weeks for the 12 weeks. Mortal fish were weighed and recorded daily .Each treatment had three replicates composed of ten snakehead fingerlings. Before the fish were weighed, the water level in the tank slowly decreased. The fish were collected using a scoop net and temporarily placed in a covered container. The fish was individually moved with a soft towel and dried using tissues and its weight and length were measured. Finally, the fish were returned to their respective tanks. At the end of each experiment, the Daily Weight Gain (DWG), Daily Length Gain (DLG), Specific Growth

Rate (SGR), Feed Intake (FI), Protein Efficiency Rate (PER), Food Conversion Ratio (FCR), Survival Rate (SR), Economic Conversion Ratio (ECR) and cost reduction were calculated using the following formulas:

- DWG (g/day) = (final weight-initial weight)/number of days
- DLG (cm/day) = (final length-initial length)/number of days
- SGR (%/day) = (ln final weight-ln initial weight(/number of days × 100
- FI (g/fish/day) = (weight of feed consumed/number of fish)/number of days
- PER = (final weight-initial weight)/consumed protein weight
- FCR = total feed consumption/fish weight gain
- SR (%) = (final fish number/initial fish number) × 100
- ECR = FCR × feed cost
- Cost reduction vs. FM™ (%) = ($ECR_{Treatment}$ - ECR_{FM}™)/ECR_{FM}™ × 100

Analysis of Diet Composition

The moisture, protein, lipid, fiber, ash, and nitrogen-free extracts of the experimental diets were determined at the beginning and end of each experiment. For moisture determination, dried samples and a ceramic beaker were weighed, then dried in an oven at 105 °C for over 4 h until a stable weight was obtained. The moisture content was evaluated from the drying loss. The sample was then incinerated at 600°C for 6–8 h. The incineration loss indicated the total ash. The fiber content was analyzed by digesting a nonfat sample with boiling sulfuric acid (0.128 M; 1.25%, w/v) followed by sodium hydroxide (0.313 m) in a fiber analyzer (Vela Scientific, Italy). The remainder was dehydrated at 100°C. The fiber content was the residue following the removal of ash. The crude protein was evaluated with the Kjeldahl technique. The crude lipids were separated from the dried sample with petroleum ether at 60°C for 8 h using Soxhlet extraction. The nitrogen-free extract was determined as [100 - (moisture + protein +lipid +fiber + ash(].

Data Analysis

The data on survival rate, growth rate, and feed utilization among the three feeding treatment groups were analyzed using a one-way Analysis of Variance (ANOVA) with multiple comparisons followed by Duncan's test to evaluate the differences among treatments at a statistical significance of P<0.05.

Results

Survival, Growth Performance and Economic Analysis

After the completion of the experiment, the survival rates were high (80.0–83.33%) and no significant differences were observed (Table 2). The final weight, weight gain, and daily weight gain of fish in the CM dietary treatment group were significantly lower than those of the fish in the SM and FM™ dietary treatment groups, but there was no significant difference between the SM and FM™ dietary treatment groups (Table 2). The specific growth rate of fish fed the CM diet was slightly less than those receiving the SM and FM™ diets .All diets resulted in continuously increased growth for C. Striata fingerlings from the second to the twelfth week of the study period (Fig. 1).

The cost reduction of producing 1 kg of fish from the diet influenced by local wisdom, containing SM or CM, compared to the FM™ diet, decreased by 7.9 and 11.6%, respectively (Table 2). However, the CM diet induced the lowest FCR in this study. FCR values and PER values among the treatment groups were not significantly different (Table 2).

Composition of Feed Diets

The composition of different diets showed significant differences in moisture and fiber contents (Table 3). The crude protein and ash contents were not significantly different among different diets. The crude lipid composition of the FM™ diet was slightly higher than that of the SM and CM diets (Table 3).

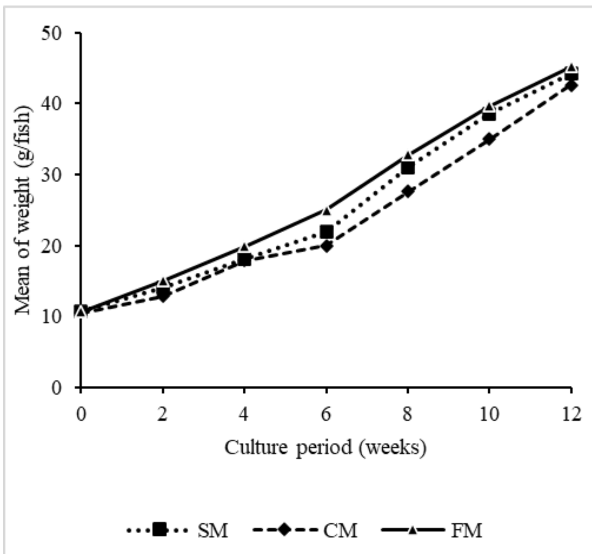

Fig. 1. Change in *C. striata* weight during 12 weeks of culturing

Table 1: Formulation of the two local wisdom feed diets (%, dry matter basis)

Ingredients	SM	CM
Soybean meal	20	-
Cassava leaf meal	-	20
Golden apple snail	18	18
Rice bran	16	16
Crushed corn	9	9
Coconut meal	9	9
Sticky rice	9	9
Acacia leaf	9	9
Banana	8	8
Premix[1]	1	1
Baking powder	1	1
Total	100	100
Total	100	100

SM = local wisdom-influenced formulation containing soybean meal; CM = local wisdom-influenced formulation containing cassava meal. [1]Premix = Vitamin and mineral premix per kg of diet: 15,000 IU Vitamin A; 2.5 mg Vitamin B1; 7 mg Vitamin B2; 4.5 mg Vitamin B6; 0.025 mg Vitamin B12; 3,000 IU Vitamin D3; 25 IU Vitamin E; 0.08 g Vitamin Fe; 0.4 mg I; 30.5 g K; 0.025 mg biotin; choline 0.25 g chloride; 1.6 mg Cu; 0.5 mg folic acid; 0.06 g Mn; 35 mg nicotinic acid; 35 mg pantothenic acid; 0.15 mg Se; and 0.045 g Zn

Table 2: Initial weight ,final weight ,daily weight gain ,initial length ,final length, daily length gain, specific growth rate, feed intake, protein efficiency rate, food conversion ratio, survival rate, feed cost, and cost reduction

Parameters	SM	CM	FM™
Initial weight (g/fish)	10.700±0.95^a	10.500±1.08^a	.1062±0.80^a
Final weight (g/fish)	44.300±0.54^a	42.590±07.5^b	452.4000±1.11^a
Daily Weight Gain (DWG) (g/day)	0.280±0.01^a	0.270±0.01^b	0.2900±0.01^a
Initial length (cm/fish)	10.590±0.72^a	10.490±0.22^a	10.1700±1.04^a
Final length (cm/fish)	18.620±0.88^a	17.380±2.37^b	17.9900±1.62^a
Daily Length Gain (DLG) (cm/day)	0.070±0.01^a	0.060±0.02^b	0.0700±0.02^a
Specific Growth Rate (SGR) (%/day)	0.790±0.03	0.780±0.03	0.8000±0.02
Feed Intake (FI) (g/fish/day)	0.370±0.27	0.390±0.28	0.3700±0.26
Protein Efficiency Rate (PER)	1.880±0.65	1.860±0.73	1.9300±0.67
Food Conversion Ratio (FCR)	1.320±0.40	1.390±0.46	1.2800±0.39
Survival Rate (SR) (%)	81.670± 2.89	80.000±5.0	83.3300±2.88
Feed cost ($/kg fish)	0.972	0.886	1.0940
Economic Conversion Ratio (ECR)	1.283	1.232	1.3930
Cost reduction vs. FM™ (%)	7.900	11.600	-

Notes: Data from three replicates are presented as mean ± SD values. This means in the same row with the different superscripts are significantly different (P<0.05). SM = local wisdom-influenced formulation containing soybean meal; CM = local wisdom-influenced formulation containing cassava meal; FM™ = commercial diet; feed cost = cost for producing 1 kg feed ($/kg feed); (economic conversion ratio) ECR (= cost/kg fish ($); cost reduction = percentage reduction in $/kg fish in comparison to FM™ feed; 1 USD = 32 THB

Table 3: Composition of the two local wisdom-influenced diets (SM, CM) and commercial feed-based diets FM™

Proximate composition (%)	SM	CM	FM™
Moisture	2.91	4.44	5.31
Crude protein	44.36	43.77	44.73
Crude lipid	8.81	8.63	9.25
Crude fiber	5.51	5.07	1.49
Ash	11.20	11.10	11.60
Nitrogen free extract	27.21	26.99	27.62

SM = local wisdom-influenced formulation containing soybean meal; CM = local wisdom-influenced formulation containing cassava meal; FM™ (Control) = commercial diet .Moisture: 11.7%, crude protein :65.1%, crude lipid :7.94%, crude fiber :0.55% and ash:13.7%

Discussion

Using soybean in feed resulted in a good performance in both fish survival and growth compared to commercial diets. The present results on growth performance were similar to those reporting the effects of a similar formulation containing 20% SM on the growth performance of *C. striata* and *C. micropellets* (Hien *et al*., 2016). Hien *et al* . (2016) replaced the fish meal with soybean in the diets of *C. striata* and *C. micropellets* and showed that fish consuming the diet containing 20% SM showed higher growth and lower cost than fish fed a formulated diet (43% protein and 12% lipids). The SM had high-protein content, a relatively balanced amino acid content, and a high digestibility profile (Hien *et al*., 2016). In this study, both formulations influenced by local wisdom, SM and CM, contained rice bran, a rich source of protein, oil, dietary fiber, and micronutrients (Hien *et al*., 2020). Rice bran is an abundant crop by-product in Thailand, the use of which resulted in reduced-cost fish feed and reduced use of trash fish. Hien *et al*. (2020) have demonstrated rice bran supplementation in feed for tilapia (*Oreochromis niloticus*) and striped catfish (*Pangasius hypophthalmus*), resulting in growth enhancement and feeding cost reduction. The widespread forms of rice bran and cassava meal are dried rice bran, wet rice bran, free-fat rice bran, and dry slashed cassava meal (Hien *et al*., 2020).

Studies of other species have shown different maximum substitution levels of fish meal with plant protein (e.g., soybean, cassava, sunflower, cottonseed, linseed, sesame, and canola meals) with no significant reduction of growth performance from the FM diet, such as the replacement of 27.3% of the FM in the diet of silvery-black porgy (*Sparidentex hasta*) (Yaghoubi *et al*., 2016), 40% in Chinese sucker (*Myxocyprinus asiaticus*) Yu *et al*. (2013), 48% in northern snakehead (*Channa argus*) (Sagada *et al*., 2017) and 83 %in striped bass hybrids (*Morone chrysops* × *Morone saxatilis*) (Rombenso *et al*., 2013). The replacement of FM with local plant sources (corn gluten meal, wheat gluten meal, SM, and bagasse kenna mix) achieved better growth performance in *Oreochromis niloticus* (Al-Thobaiti *et al*., 2017). However, Miao *et al*. (2018) have revealed that northern snakeheads fed more than 50% of dietary SM contained a smaller relative amount of lactic acid bacteria, a greater number of opportunistic diseases, and up-regulated inflammatory cytokine gene expression of the intestinal cells. SM is the principal protein source from the plant for extensive use in aquaculture feed. It is one of the most suitable choices for fish meal substitution because it has high protein content, excellent amino acid composition, and a high level of digestibility.

In this study, the local wisdom-influenced formulations, SM and CM, for *C. striata*, had an economic advantage. The maximal cost reduction for producing 1 kg of fish was observed in the CM diet (11.6%), followed by the SM diet (7.9%). CM diet induced the lowest FCR in this experiment. However, the FCR value found in this study of three experimental groups (1.28-1.39) was in between the typical FCR value (1.0–2.4) for farmed fish (Fry *et al*., 2018). Utilization of a plant-protein diet is now in demand, particularly for carnivorous fish to diminish dependence on fish meal, feed cost, and environmental impact .Replacing the fish meal with a plant-protein source diet is advantageous for reducing the cost of feed and decreasing demand for fish meal supplements in grouper (*Epinephelus fuscoguttatus*), a carnivorous fish. There was no significant difference between feeding a combined recipe of a 1:1 formulated diet to trash fish and a diet of only trash fish (Rachmansyah *et al*., 2009).

Conclusion

This study demonstrated the efficacy of plant-protein source diets .Fish feed formulated with plant-protein source feed had a positive effect, particularly when supplemented with SM, which resulted in elevated fish growth with small FCR and great PER, comparable to the commercial diet. The compositional analysis indicated that the feed formulation influenced by local wisdom containing plant protein had protein and lipid contents as high as the commercial feed . Consequently, the reduction in the use of the fishmeal diet could be enhanced by using the local wisdom-influenced feed formulations based on the data obtained from this research study, to directly affect the sustainability of living resources by reducing the demand for fisheries. Moreover, further study of the local wisdom-influenced diet for snakehead fish should consider fish immunity and possible abnormalities in fish such as lordosis and scoliosis that were reported in fish-fed plant-protein source feed (Hien *et al*., 2016).

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Author’s Contributions

Duangjai Boonkusol: Designed the experiment, performed the study, analyzed and interpreted the study findings, drew conclusions, and contributed to manuscript preparation and revisions.

Wuttipong Tongbai: Coordinated the implementation of research, compiled the literature review, and contributed to manuscript revisions.

Ethics

This article is original and contains unpublished material .The corresponding author confirms that all of the other authors have read and approved the manuscript and there are no ethical issues.

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